

*What Is Claimed Is:*

1. A method of inhibiting or preventing pyrophosphorolysis during synthesis of a nucleic acid molecule, said method comprising

(a) combining one or more nucleotides and a nucleic acid template; and

(b) incubating the one or more nucleotides and nucleic acid template together with a polymerase and an enzyme selected from the group consisting of a pentosyltransferase, a phosphotransferase with alcohol group as acceptor, a nucleotidyltransferase, and a carboxy-lyase, under conditions sufficient to form a second nucleic acid molecule complementary to all or a portion of the nucleic acid template.

2. A method of inhibiting or preventing pyrophosphorolysis during synthesis of a nucleic acid molecule, said method comprising

(a) combining a primer with a nucleic acid template under conditions sufficient to form a hybridized product; and

(b) incubating said hybridized product in the presence of (i) one or more nucleotides, (ii) a polymerase, and (iii) an enzyme selected from the group consisting of a pentosyltransferase, a phosphotransferase with an alcohol group as acceptor, a nucleotidyltransferase, and a carboxy-lyase under conditions sufficient to synthesize a second nucleic acid molecule complementary to all or a portion of said nucleic acid template.

3. The method of claim 2, wherein said enzyme of (b)(iii) is a pentosyltransferase.

4. The method of claim 3, wherein said enzyme is an adenine phosphoribosyltransferase or an orotate phosphoribosyltransferase.

5. The method of claim 2, wherein said enzyme of (b)(iii) is a phosphotransferase with an alcohol group as acceptor.

6. The method of claim 5, wherein said enzyme is a pyrophosphate: glycerol phosphotransferase, a pyrophosphate: serine phosphotransferase, a pyrophosphate: fructose-6-phosphate 1-phosphotransferase or a pyrophosphate: purine nucleoside kinase.

7. The method of claim 2, wherein said enzyme of (b)(iii) is a nucleotidyltransferase.

8. The method of claim 7, wherein said enzyme is an ATP: sulfate adenylyltransferase, a UTP: glucose-1-phosphate uridylyltransferase or an ATP: glucose-1-phosphate adenylyltransferase.

9. The method of claim 2, wherein said enzyme of (b)(iii) is a carboxy-lyase.

10. The method of claim 9, wherein said enzyme is a phosphoenolpyruvate carboxykinase.

11. The method of claim 2, wherein said enzyme of (b)(iii) is a thermostable enzyme.

12. The method of claim 2, wherein said nucleotide is a deoxyribonucleoside triphosphate and said polymerase is a DNA polymerase.

13. The method of claim 1, wherein said nucleotide is a ribonucleoside triphosphate and said polymerase is an RNA polymerase.

14. A method to prevent inhibition of nucleic acid synthesis during amplification of a double stranded nucleic acid molecule, comprising

5 (a) providing a first and second primer, wherein said first primer is complementary to a sequence at or near the 3' termini of the first strand of said nucleic acid molecule and said second primer is complementary to a sequence at or near the 3' termini of the second strand of said nucleic acid molecule;

10 (b) hybridizing said first primer to said first strand and said second primer to said second strand in the presence of (i) a polymerase, and (ii) an enzyme selected from the group consisting of a pentosyltransferase, a phosphotransferase with an alcohol group as an acceptor, a nucleotidyltransferase and a carboxy-lyase under conditions such that a third nucleic acid molecule complementary to said first strand and a fourth nucleic acid molecule complementary to said second strand are synthesized;

15 (c) denaturing said first and third strand and said second and fourth strand; and

(d) repeating steps (a) to (c) one or more times.

15. The method of claim 14, wherein said enzyme of (b)(ii) is a pentosyltransferase.

20 16. The method of claim 15, wherein said enzyme is an adenine phosphoribosyltransferase or an orotate phosphoribosyltransferase.

17. The method of claim 14, wherein said enzyme of (b)(ii) is a phosphotransferase with an alcohol group as acceptor.

25 18. The method of claim 17, wherein said enzyme is a pyrophosphate: glycerol phosphotransferase, a pyrophosphate: serine phosphotransferase, a pyrophosphate: fructose-6-phosphate 1-phosphotransferase or a pyrophosphate: purine nucleoside kinase.

19. The method of claim 14, wherein said enzyme of (b)(ii) is a nucleotidyltransferase.

20. The method of claim 19, wherein said enzyme is an ATP: sulfate adenylyltransferase, a UTP: glucose-1-phosphate uridylyltransferase or an ATP: glucose-1-phosphate adenylyltransferase.

21. The method of claim 14, wherein said enzyme of (b)(ii) is a carboxy-lyase.

22. The method of claim 21, wherein said enzyme is a phosphoenolpyruvate carboxykinase.

23. The method of claim 14, wherein said enzyme of (b)(ii) is a thermostable enzyme.

24. The method of claim 14, wherein said polymerase is a DNA polymerase.

25. A method of sequencing a DNA molecule comprising:

(a) combining a primer with a first DNA molecule under conditions sufficient to form a hybridized product;

(b) contacting said hybridized product with (i) nucleotides; (ii) a DNA polymerase; (iii) an enzyme selected from the group consisting of a pentosyltransferase, a phosphotransferase with an alcohol group as acceptor, a nucleotidyltransferase and a carboxy-lyase; and a terminator nucleotide to give a reaction mixture;

(c) incubating the reaction mixture under conditions sufficient to synthesize a random population of DNA molecules complementary to said first DNA molecule, wherein said synthesized DNA molecules are shorter in length

than said first DNA molecule and wherein said synthesized DNA molecules comprise a terminator nucleotide at their 3' termini; and

(d) separating said synthesized DNA molecules by size so that at least a part of the nucleotide sequence of said first DNA molecule can be determined.

5           26. The method of claim 25, wherein said enzyme of (b)(iii) is a pentosyltransferase.

27. The method of claim 26, wherein said enzyme is an adenine phosphoribosyltransferase or an orotate phosphoribosyltransferase.

10           28. The method of claim 25, wherein said enzyme of (b)(iii) is a phosphotransferase with an alcohol group as acceptor.

29. The method of claim 28, wherein said enzyme is a pyrophosphate: glycerol phosphotransferase, a pyrophosphate: serine phosphotransferase, a pyrophosphate: fructose-6-phosphate 1-phosphotransferase or a pyrophosphate: purine nucleoside kinase.

15           30. The method of claim 25, wherein said enzyme of (b)(iii) is a nucleotidyltransferase.

31. The method of claim 30, wherein said enzyme is an ATP: sulfate adenylyltransferase, a UTP: glucose-1-phosphate uridylyltransferase or an ATP: glucose-1-phosphate adenylyltransferase.

20           32. The method of claim 25, wherein said enzyme of (b)(iii) is a carboxy-lyase.

33. The method of claim 32, wherein said enzyme is a phosphoenolpyruvate carboxykinase.

34. The method of claim 25, wherein said enzyme of (b)(iii) is a thermostable enzyme.

5 35. The method of claim 25, wherein said nucleotides are deoxyribonucleoside triphosphates and said polymerase is a DNA polymerase.

36. A solution for use in nucleic acid synthesis, amplification or sequencing, comprising

10 (a) an enzyme selected from the group consisting of a pentosyltransferase, a phosphotransferase with alcohol group as acceptor, a nucleotidyltransferase, and a carboxy-lyase;

(b) a substrate which is capable of either accepting either a phosphate radical to give a phosphorylated product from pyrophosphate or effecting transfer of pyrophosphate when in the presence of said enzyme; and

15 (c) a polymerase.

37. The solution of claim 36, wherein said enzyme of (a) is a pentosyltransferase.

38. The solution of claim 37, wherein said enzyme is an adenine phosphoribosyltransferase or an orotate phosphoribosyltransferase.

20 39. The solution of claim 36, wherein said enzyme of (a) is a phosphotransferase with an alcohol group as acceptor.

40. The solution of claim 39, wherein said enzyme is a pyrophosphate: glycerol phosphotransferase, a pyrophosphate: serine phosphotransferase, a

pyrophosphate: fructose-6-phosphate 1-phosphotransferase or a pyrophosphate: purine nucleoside kinase.

41. The solution of claim 36, wherein said enzyme of (a) is a nucleotidyltransferase.

5 42. The solution of claim 41, wherein said enzyme is an ATP: sulfate adenylyltransferase, a UTP: glucose-1-phosphate uridylyltransferase or an ATP: glucose-1-phosphate adenylyltransferase.

43. The solution of claim 36, wherein said enzyme of (a) is a carboxy-lyase.

10 44. The solution of claim 43, wherein said enzyme is a phosphoenolpyruvate carboxykinase.

45. The solution of claim 36, wherein said enzyme of (a) is a thermostable enzyme.

15 46. The solution of claim 36, wherein said polymerase is a DNA polymerase.

47. The solution of claim 36, wherein said polymerase is an RNA polymerase.

20 48. A kit comprising a container means having in close confinement therein two or more container means, wherein a first container means comprises an enzyme selected from the group consisting of a pentosyltransferase, a phosphotransferase with an alcohol group as acceptor, a nucleotidyltransferase, and a carboxy-lyase; and a third container means contains a substrate which is

capable of either accepting a phosphate radical to give a phosphorylated product from pyrophosphate or effecting transfer of pyrophosphate when in the presence of said enzyme;

wherein a nucleic acid polymerase is optionally present in said first container means or is optionally comprised in a second container means.

49. The kit of claim 48, wherein said nucleic acid polymerase is present in said first container means.

50. The kit of claim 48, wherein said nucleic acid polymerase is present in said second container means.

51. The kit of claim 48, wherein said enzyme is a thermostable enzyme.